Total Fatty Acid Analysis of Vegetable Oil Soapstocks by Supercritical Fluid Extraction/Reaction

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ABSTRACT: Soapstock from vegetable oil refining operations is a value-added by-product that finds further industrial use based on its fatty acid content. Since the fatty acid content of soapstock can vary according to its vegetable oil source or method of refining, determination of its total fatty acid (TFA) by an accurate analytical method is of key importance to purchasers of this refinery by-product. Traditionally, the TFA content of soapstock has been determined by the AOCS Official Method G3-53 based on a gravimetric assay. Unfortunately, this gravimetric-based assay requires considerable time and incorporates a considerable quantity of organic solvent per assay. In this study, the authors have applied supercritical fluid extraction (SFE) with an enzymatic-based reaction (SFR), in the presence of supercritical carbon dioxide (SC-CO_2), to determine the TFA content of soapstocks. The SFE/SFR sequence was conducted using two commercially available extractors using an *in situ* supported lipase in the extraction cell to form fatty acid methyl esters (FAME). Gas chromatographic (GC) determination of the individual FAME, followed by quantitation based on the calculated sum of all the fatty acids from the GC analysis, allowed a precise determination to be made of the soapstock's TFA content. The TFA contents of three different soapstocks determined by this method were slightly higher than the values derived from Official Method G3-53. The reported method takes less than one-half of the time of Official Method G3-53 and reduces organic solvent use from 575 mL to under 2 mL of solvent by using SC-CO_2.

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KEY WORDS: Carbon dioxide, extraction, reaction, soapstock, supercritical fluid.

Soapstock from the vegetable oil processing industry is purchased by industrial companies for a variety of end uses. Historically, many of these uses have been based on its fatty acid content (1), but additional cited applications of soapstock include its use as a feed (2), feedstock for chemical reactions (3–5), nutrient source for microorganisms (6), fertilizer ingredient (7), and potential application as a methylated herbicidal adjuvant (8). Since the fatty acid content of the soapstock can vary according to the vegetable oil source and method of refining (9,10), an accurate quantitative analysis of its total fatty acid content is critical to purchasers of this product.

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The by-product from the oil refining process is delivered to companies *via* tankers, hence a rapid analysis of the total fatty acid content of each soapstock shipment is critical to the product purchasers. The soapstock fatty acid content has traditionally been assessed using a solvent extraction/gravimetric assay-based procedure that can take 5–8 h to perform (11). Such a lengthy analysis can result in additional demurrage charges on the delivery vehicle while the analysis is performed.

Supercritical-fluid-based extraction and chromatographic methods (12–17) are becoming increasingly popular, particularly those that employ environmentally benign supercritical carbon dioxide (SC-CO_2). This interest is due to the time savings involved when substituting SC-CO_2 for a liquid solvent and the reduction and/or elimination of organic solvents used in traditional assays (18,19). Recently, the American Oil Chemists' Society approved an official method to determine oil in oilseeds *via* supercritical fluid extraction (SFE) (20,21). For similar reasons, supercritical fluid chromatography (SFC) is also being used more frequently for rapid proximate analysis of oleochemicals (22) as well as in support of research and development studies (23).

In this study the authors have utilized SFE with SC-CO_2, coupled with an enzymatically catalyzed reaction in SC-CO_2 (SFR) to assess the fatty acid content of soapstock more rapidly (24). The authors previously used this method to determine the oil and/or fat content of foodstuffs (25) based on research involving fatty acid methyl ester (FAME) formation in supercritical fluid media (26). This method is more specific for the fatty acid moiety, since it provides a detailed gas chromatographic analysis of the fatty acid profile in the soapstock as opposed to a nonspecific assay, such as AOCS Method G3-53 (11). Method G3-53 has been utilized for many years to approximate the total fatty acid content of oil-based products (10), but like many titrimetric-based methods, it is influenced by interferences when conducting the assay (27).

MATERIALS AND METHODS

Soapstock samples. Three different soapstock samples were utilized in this study. Two were derived from soybean feedstock, while the other was a corn-oil-based soapstock. Soybean-based soapstocks were supplied by Agrotech, Inc. (Sherman, TX) and Central Soya, Inc. (Decatur, IN). The corn-oil-based soapstock was provided by Anderson Clay-

ton/Humko Products, Inc. (Memphis, TN). Each lot of soapstock was divided into 1 L aliquots and stored at −29°C until use. During the course of experimentation, the soapstock aliquots were stored at 2°C. Prior to SFE/SFR, the soapstock (0.1–0.2 g) was combined with 0.4 g Chem-Tube Hydromatrix (Analytichem International, Harbor City, CA) and lyophilized using an FTS Systems Flexi-dry freeze dryer (Stone Ridge, NY) for 1 h.

Immobilized lipase. Novozyme 435 was obtained from Novo Nordisk (Danbury, CT). The immobilized enzyme is described by the manufacturer as containing 1–2% water by weight and having 7000 units/g activity in terms of propyl laurate synthesis. Prior to SFE/SFR, the lipase (1.5 g) was placed in an extraction cell (7 mL for the Hewlett-Packard and 10 mL for the Isco), between glass wool plugs, and conditioned with CO_2 at 17.6 MPa and 50°C with a flow rate of 1 mL/min for 20 min, then held there statically for 0.3 min prior to initiating dynamic SFE. During this period of time, the restrictor was heated to 65°C.

SFE/SFR. SFE/SFR was performed utilizing two different commercially available instruments: (i) a Hewlett-Packard Model 7680T (Hewlett-Packard, Wilmington, DE) and (ii) an Isco Model SFX 3560 (Isco Inc., Lincoln, NB). After the enzyme was conditioned as described previously, triundecanoin (0.5 mg) was added to the extraction/reaction cell as an internal standard. The soapstock–Hydromatrix mix was then added to the extraction cell upstream of the enzyme bed and the remaining volume in the extraction vessel was filled with glass wool. The extraction/reaction sequence then proceeded under the following conditions: 19.7 MPa, 50°C, 1 mL/min, 80 min, 1% modifier (vol/vol) with the restrictor held at 65°C. A 1:1 methanol/hexane mixture (25) was used as a modifier in SC-CO_2 to provide methanol to form the FAME. Since the Model 7680T utilizes a C-18 trap to isolate the extracting/reacting solutes, it was held at 30°C for collection purposes. The C-18 trap on the Model 7680T was rinsed with hexane (1.0 mL) to transfer the extracted/reacted solutes into a 1.8-mL autosampler vial (trap temperature = 50°C). The Model SFX-3560 employed a liquid-based collection sequence in which a vial containing hexane (10 mL, cooled to 5°C) was used to absorb the solutes from the depressurized CO_2 stream. The solvent, in the case of the Isco collection vial, was concentrated to dryness under a nitrogen stream, reconstituted to 1 mL, and then transferred to a 1.8-mL autosampler vial.

SFC. SFC was performed on a Lee Scientific Series Model 600 supercritical fluid chromatograph (Dionex Corporation, Sunnyvale, CA) equipped with a 200-nL injection loop and a flame ionization detector (FID) held at 350°C. A Dionex SB-Phenyl-50 capillary column (10 m × 50 μm i.d., 0.25 μm film thickness), held at 100°C, was used with the following pressure gradient: 10.1 MPa for 5 min, then increased at 0.4 MPa/min to 24.2 MPa, followed by an increase of 1.0 MPa/min to 32.4 MPa. SFE/SFR-grade CO_2 (Air Products and Chemicals, Inc., Allentown, PA) was the carrier fluid. Chromatograms were analyzed with a Data Jet integrator (Spectra-Physics Analytical, San Jose, CA). Standards of methyl palmitate, palmitic acid, monopalmitin, dipalmitin, and tripalmitin were used as is to determine the response factors for the FID. Methyl palmitate was used as the internal standard.

Analysis of SFE/SFR extracted products. Total fatty acid content was determined from the analysis of the resulting FAME using a Hewlett-Packard Model 5890 Series II gas chromatograph (Palo Alto, CA) incorporating a Supelco SP-2340 (60 m × 0.25 mm, 0.2 μm film thickness; Bellefonte, PA) column. The injector and FID temperatures were 235 and 250°C, respectively. The oven temperature was held at 100°C for 5 min and then programmed to 200°C at 3°C/min. Helium was the carrier gas at a flow rate of 1 mL/min. Column head pressure was held constant at 0.14 MPa.

Gravimetric fatty acid determination. Total fatty acid content of the soapstocks was quantitated by gravimetric analysis according to AOCS Official Method G3-53 (26).

RESULTS AND DISCUSSION

To show that the described method was not instrumentally biased, soybean-based soapstock #1 was subjected to the SFE/SFR sequence performed in triplicate on both the Model SFX 3560 and Model 7680T extractors. As can be seen in Table 1, the results obtained on the two different extractors appear to be identical; this is confirmed using a general analysis of variance, ANOVA, and LSD (T), least significant differences, pairwise comparison using Statistix 4.1 program (Analytical Software Co., Tallahassee, FL). Comparison of the individual analysis values and resultant means for the SFE/SFR results with those obtained from AOCS Method G3-53 indicates that the AOCS method gives lower values for the total free fatty acid content on this particular soapstock. This was also confirmed using the statistical comparison tests mentioned previously. The relative standard deviations (RSD) associated with determinations performed on both instruments are similar in magnitude to that found when applying Method G3-53, between 1–3 % RSD.

Data for similar assays run on the Model SFX 3560 instrument for soybean-based soapstock #2 and the corn-oil-derived soapstock showed similar trends to those noted previously (Tables 2 and 3). Again the mean values for total fatty

TABLE 1
Total Fatty Acid Content of Soybean-Based Soapstock #1[a]

AOCS Method G3-53 (ref. 11)	SFE/SFR	
	SFX-3560	HP 7680T
30.56	32.40	32.85
31.52	32.87	31.26
31.08	32.62	32.84
Avg. 31.05	32.63	32.32
SD 0.48	0.24	0.92
RSD 1.55	0.72	2.83

[a]All data in percentages. SFE/SFR, supercritical fluid extraction coupled with an enzymatically catalyzed reaction in supercritical CO_2; RSD, relative standard deviation; SFX-3560, SFE/SFR manufactured by Isco Inc., Lincoln, NB; HP 7680T, SFE/SFR manufactured by Hewlett-Packard, Wilmington, DE.

TABLE 2
Total Fatty Acid Content of Soybean-Based Soapstock #2[a]

AOCS Method G3-53	SFE/SFR
11.01	11.92
11.04	12.04
11.05	12.87
Avg. 11.03	12.28
SD 0.02	0.07
RSD 0.19	0.60

[a]All data in percentages. For abbreviation see Table 1.

TABLE 3
Total Fatty Acid Content of Corn Oil-Based Soapstock[a]

AOCS Method G3-53	SFE/SFR
38.33	39.92
39.83	39.38
38.35	39.27
Avg. 38.84	39.52
SD 0.86	0.33
RSD 2.22	0.84

[a]All data in percentages. For abbreviation see Table 1.

acid content based on triplicate determinations were slightly higher when using the SFE/SFR method. The precision associated with the SFE/SFR soapstock method in these latter two cases is very high, less than 1% RSD. It is interesting to note that substantially different values of total free fatty acid were found for the three soapstocks, two of which are soy based. This was confirmed by the ANOVA and LSD (T) pairwise comparison of the means. Statistical analysis also showed that there was no interaction between the extraction method applied and the soapstock source. Knowledge of the difference in the free fatty acid content of individual soapstocks is critical to soapstock purchasers who require such data when accepting shipments of this material.

As will be noted shortly, the SFE/SFR can be performed in approximately one-half of the time of the AOCS method. The authors have also utilized SFC to determine the approxi-

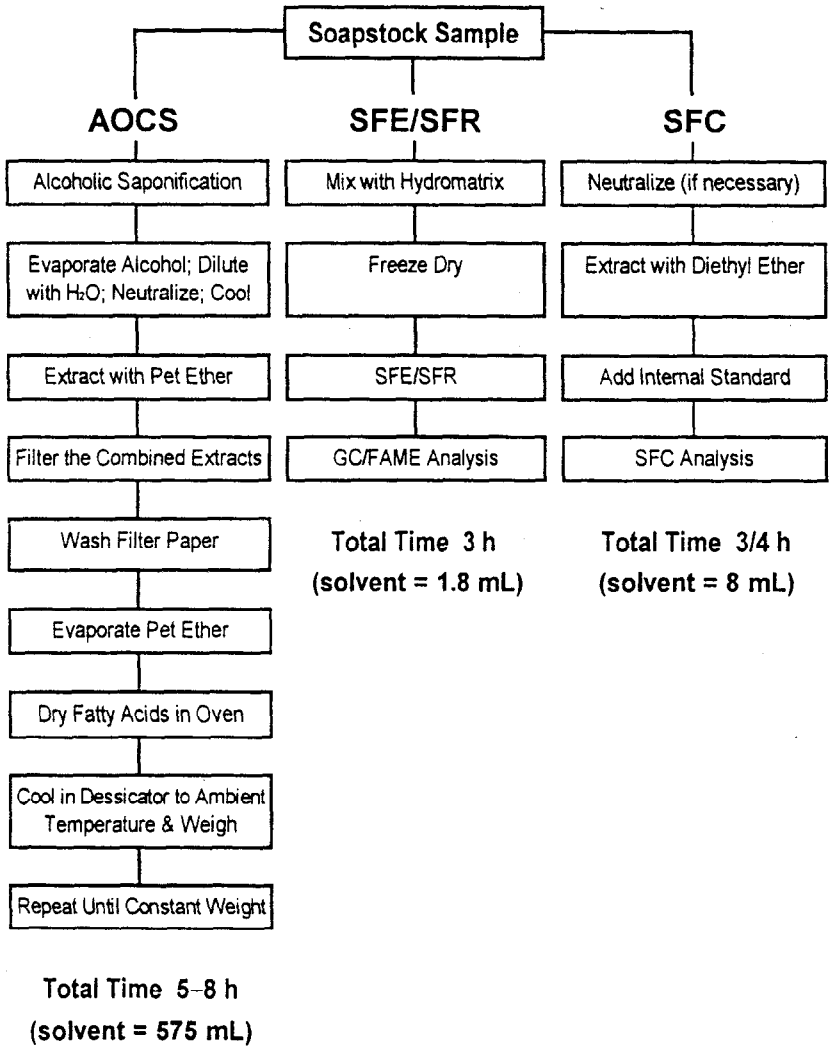

FIG. 1. Comparison of AOCS Official Method G3-53 (ref. 11) with the supercritical fluid extraction coupled with an enzymatically catalyzed reaction (SFE/SFR) and supercritical fluid chromatography (SFC) methods. Abbreviations: GC, gas chromatography; FAME, fatty acid methyl ester. Hydromatrix (Analytichem International, Harbor City, CA).

TABLE 4
Total Fatty Acid Analysis: AOCS Method G3-53 vs. SFC Method[a]

Soapstock sample	AOCS Method G3-53[b]	SFC method
Soybean #1	31.05 (1.55)	29.16 (2.77)
Soybean #2	11.03 (0.19)	8.75 (5.63)
Corn	38.84 (2.22)	37.30 (3.02)

[a]All data in percentages, $n = 3$.
[b]Numbers in parentheses represent relative standard deviation. For abbreviation see Table 1.

mate free fatty acid content of the three soapstocks. This technique, like the SFE/SFR, has the potential of reducing analysis time and solvent use. Results from the SFC method are shown in Table 4 where they are compared with the total free fatty acid means from AOCS Method G3-53. In this case there is a decided bias toward a lower total free fatty acid value when using SFC vs. the gravimetrically based Method G3-53. There is also a higher RSD associated with the determinations *via* the SFC. This would seem to indicate that the SFC method should be rejected outright, however the SFC method requires only 3/4 h per sample. Hence, for proximate analysis purposes, the SFC method, which requires only 8 mL of solvent, may have value for rapidly estimating the free fatty acid level in soapstocks.

A comparison of the complexity of the three methods is noted in Figure 1. Here it can be seen that the AOCS Official Method G3-53 requires a number of discrete, analyst-dependent steps to assess the free fatty acid content of individual soapstocks. Depending on the speed and skill of the analyst, the total analysis time using Method G3-53 is estimated to be 5–8 h. A distinct disadvantage of this method is the large amount of solvent required per analysis: 575 mL. This may be compared with the SFE/SFR method, which requires only 1.8 mL solvent per assay. Using this analytical technique, we estimate that each assay will take only 3 h to complete. The previously mentioned SFC method, despite its inherent inaccuracy, takes an even shorter time to perform, approximately 45 min, and incorporates only 8 mL organic solvent. As noted in Figure 1, both the SFE/SFR and SFC methods are less complex in terms of the number of sequential steps required. The lipid specificity (24) of the SFE/SFR method, the authors believe, provides a more accurate determination of the free fatty acid content of soapstocks and is not subject to the errors associated with the nonspecific, gravimetric-based Method G3-53 assay.

In summary, the authors believe the two supercritical fluid-based methods (SFE/SFR and SFC) deserve serious consideration to determine the fatty acid content of commercial soapstocks. Both methods substantially eliminate the use of solvents in the laboratory and, as demonstrated, can be performed in less time. The SFE/SFR method also has the added advantage that it can be run overnight owing to the automated features of the SFE instrumentation. Although the SFC method appears to be less accurate and precise than the gravimetric or SFE/SFR methods, it has the advantage of speed and can be used to give a semiquantitative estimate of the free fatty acid content and to detect other major lipid species in the soapstock. For example, the SFC method permits one to assess the glyceride composition of the soapstock without resorting to additional analytical methods. Others have recently shown an interest in characterizing the total molecular composition of soapstock, such as the low molecular weight composition of acid waters (28–30) Such data may be sufficient to pass judgment on the acceptance or rejection of a shipment of soapstock at a processing plant.

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